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Synthesis, structure and antibacterial activity of some triorganotin(IV) complexes with a benzamidoalanine ligand

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Abstract

New triorganotin(IV) complexes of the type $PhSnL_3$, $BuSnL_3$ and $MeSnL_3$ were constructed with the ligand benzamidoalanine, which was formed by reaction of benzoyl chloride with alanine in the presence of sodium hydroxide. The complexes were characterized by elemental analysis, conductance measurements and infrared, ultraviolet visible and ^{1}H, ^{13}C and ^{119}Sn nuclear magnetic resonance spectroscopy. Monomer structures were proposed from the spectral measurements, with a bidentate form. All compounds were screened for antibacterial (*Staphylococcus aureus* ATCC-9144, *S. epidermidis* ATCC-155, *Micrococcus luteus* ATCC-4698, *Bacillus cereus* ATCC-11778, *Escherichia coli* ATCC-25922 and *Pseudomonas aeruginosa* ATCC-2853) and antifungal (*Aspergillus niger* ATCC-9029 and *A. fumigatus* ATCC-46645) activity by paper disc diffusion. The minimum inhibitory concentrations of the compounds were determined by agar streak dilution. $MeSnL_3$ was the most potent antimicrobial agent when compared with ciprofloxin and ketoconazole.

We describe here a simple, convenient route for synthesizing new organotin derivatives for antimicrobial evaluation.

Keywords: Triorganotin(IV); Carboxylate; Spectral studies; Antimicrobial

1. Introduction

Organotin carboxylates have attracted considerable attention because of their wide application in many fields [1–3]. The coordination number and environment of the tin atom in organotin carboxylates can be controlled by adjusting the steric and electronic factors of the carboxylic acid ligands and substituents linked to the tin atom [3,4]. Many investigations have been conducted on organotin carboxylates with functionalized carboxylic acids and additional O, S or N donor groups [5].

The structural diversity of organotin carboxylates is well recognized. In the presence of additional coordinating atoms, some organotin carboxylates have fascinating structures, such as hexameric cyclic forms [6,7], with a

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wide variety of coordination geometries [8]. It is considered that a combination of steric and electronic factors determine the specific structure adapted by a particular organotin carboxylate [9], as supported by observation of monomeric, dimeric, tetrameric, oligomeric ladder, cyclic and drum structures. Furthermore, it has been reported that the size of the carboxylic acid used and the stoichiometry of the reactants play important roles in the formation of solid state frameworks.

Organotin compounds have a wide range of applications [10], including biological activity as potential antineoplastic and anti-tuberculosis agents [11,12], plastic stabilizers [13,14] and polymer catalysts [15]. Recent studies on organotin carboxylates have involved either single crystal structure determination or spectroscopy [16]. We undertook the preparation and characterization of three organotin complexes with benzamidoalanine: triphenyltin(IV)bis(benzamidoalanine) (Ph_3SnL), tributyltin(IV)bis(benzamidoalanine) (Bu_3SnL) and trimethyltin(IV) bis(benzamidoalanine) (Me_3SnL) and determined their antimicrobial activity.

2. Materials and methods

2.1. Synthesis of benzamidoalanine

One gram (5 mmol) of alanine was dissolved in 25 ml of 5% NaOH solution in a conical flask. Benzoyl chloride (2.25 ml, 20 mmol) was added in five 0.5-ml increments, and the flask was shaken vigorously until all the chloride had reacted. After acidification with diluted hydrochloric acid (1 ml), the crude product was washed with cold ether. The desired product was recrystallized from ethanol.

2.2. Preparation of complexes

Triorganotin salts (0.199 g, 1 mmol) were added drop wise with constant stirring to a hot toluene solution of the ligand (0.193 g, 1 mmol). Then, the solution was refluxed for 6 h with a magnetic stirrer, cooled and filtered. After drying at 60 °C, the solid obtained was recrystallized in a mixture of ethanol and petroleum ether. The purity of the ligand and its complexes were checked by thin-layer chromatography with silica gel-G as the adsorbent.

2.3. Spectroscopy

Elemental C, H, N and S analysis was carried out on a Fison EA 1108 analyser, and Fourier transform infrared spectra in the range 4000–370 cm^{-1} were recorded as potassium bromide discs on a Perkin-Elmer spectrophotometer GX. Molar conductance measurements were made in anhydrous dimethylformamide at 25 °C on an Inolop-Cond Level 1 WTW. The atomic absorption spectra of the complexes were measured on a Shimadzu 680-cc spectrometer. The ^{1}H, ^{13}C and ^{119}Sn nuclear magnetic resonance spectra were recorded on a Jeol 400 MHz spectrometer, relative to the internal standard tetramethylsilane. Melting-points were determined in open capillary tubes with an Electrothermal 9300 digital apparatus.

2.4. Biological investigations

The antibacterial activity of the synthesized compounds was tested in four Gram-positive bacteria (*Staphylococcus aureus* ATCC-9144, *S. epidermidis* ATCC-155, *Micrococcus luteus* ATCC-4698 and *Bacillus cereus* ATCC-11778) and three Gram-negative bacteria (*Escherichia coli* ATCC-25922 and *Pseudomonas aeruginosa* ATCC-2853) in nutrient agar medium. The antifungal activity of the compounds was tested in *Aspergillus niger* ATCC-9029 and *A. fumigatus* ATCC-46645 in Sabouraud dextrose agar. All the fungal and mould strains were clinical isolates, characterized by conventional morphological and biochemical methods.

The medium was sterilized by autoclaving at 120 °C for 30 min, inoculated at a temperature of 40–50 °C with 1 mL/100 mL of suspensions (10^5 cfu mL^{-1}) of the microorganisms (matched to McFarland barium sulfate standard) and poured onto a petri dish to a depth of 3–4 mm. Filter paper impregnated with the test compounds (μg/mL in dimethylformamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (100 μg/disc) and ketoconazole (100 μg/disc) were used as standards for antibacterial and antifungal activity, respectively.

The minimum inhibitory concentrations (MICs), the lowest concentrations of the test substances exhibiting no visible growth of bacteria or fungi on the plate, were determined by the agar streak dilution method. Stock solutions of the synthesized compounds (100 μg/mL) in dimethylformamide were prepared, and graded quantities were incorporated into specified quantities of molten sterile nutrient agar for determination of antibacterial activity and in Sabouraud dextrose agar medium for antifungal activity.

Table 1
Physical data for ligand and organotin complexes.

Compound	Colour	Yield (%)	Melting-point (°C)	Found (calculated) (%)			
				C	H	N	Sn
Ligand	White	75	165–166	62.32 (62.17)	5.81 (5.74)	7.17 (7.25)	–
Ph_3SnL	White	83	149–151	57.65 (62.02)	4.99 (4.65)	4.12 (2.58)	17.71 (21.89)
Bu_3SnL	White	78	161–162	53.79 (54.79)	6.44 (7.73)	3.82 (2.90)	19.93 (24.62)
Me_3SnL	White	75	177–179	50.06 (43.86)	4.06 (4.92)	5.53 (3.93)	22.41 (33.35)

3. Results and discussion

3.1. Synthesis and characterization of organotin complexes

Table 1 shows the physical characteristics of the ligand and the complexes. The conductance of the complexes at room temperature was in the range 8–18 $\Omega^{-1}\,cm^2\,mol^{-1}$, suggesting a non-electrolytic nature. The calculated values for elemental C, H, N and S and tin were in a good agreement with the experimental values.

The Fourier transform infrared spectrum of the ligand showed characteristic stretching absorption bands at 3371 cm^{-1}, 3328 cm^{-1}, 1611 cm^{-1} and 1332 cm^{-1}, which were assigned to υ(OH), υ(N–H), υ(COO) asym and υ(COO) sym, respectively. The COO stretching vibrations are important for predicting the bonding mode of the ligand. The values of $\Delta\upsilon$ [$\Delta\upsilon = \upsilon$ asym (COO) − υ sym (COO)] can be divided into three groups [17]. (i) In compounds in which $\Delta\upsilon$(COO) > 350 cm^{-1}, the carboxylate group binds in a monodentate fashion, although other, very weak intra- and intermolecular interactions cannot be excluded. (ii) When $\Delta\upsilon$ (COO) < 200 cm^{-1}, the carboxylate groups of these compounds can be considered to be bidentate. (iii) In compounds in which $\Delta\upsilon$ (COO) > 200 cm^{-1} and <350 cm^{-1}, an anisobidentate state, intermediate between monodentate and bidentate, occurs. It has been suggested that the $\Delta\upsilon$ (COO) value in the chelating mode is less than that in the bridging mode. On this basis, we propose that the investigated compounds are chelating-type carboxylates. The disappearance of the hydrogen from the hydroxyl group on complexation indicates that complexation occurs at the oxygen atom. The bands for υ(Sn–C) and υ(Sn–O) were assigned at 531–556 and 443–447 cm^{-1}, respectively. The infrared data for the complexes are shown in Table 2, which lists the stretching frequencies (υ) of some of the characteristic groups in the ligand and complexes.

The ^1H NMR spectra of the compounds are shown in Table 3. The spectra support the formation of a benzamidoalanine chelate, which gives a single resonance near δ 8.71 ppm attributable to the N–H proton. The spectra also show singlet –OH peaks at δ 9.24 ppm due to a hydroxyl group. The hydroxyl resonance is absent from the spectra of the complexes, indicting deprotonation and coordination of tin to the oxygen. A small increase in the shift of the aromatic proton resonances of the ligand is seen on chelation with the triorganotin(IV) moiety [18]. The complexes Ph_2SnL_2, Bu_2SnL_2 and Me_2SnL_2 showed additional signals. Methyltin (Sn–CH_3) was seen at δ 1.35, 1.33 and 1.31 ppm as a sharp singlet at integrates for the protons, accompanied by satellites due to the ^1H–^{119}Sn coupling, which corresponds to the hydrogen atom of the methyl protons of Me–Sn for Me_2SnL_2. In the dibutyltin(IV) complex, the butyl protons appear as a multiple and a triplet in the range δ 1.55–0.72 ppm

Table 2
Characteristic absorption bands of ligand and complexes.

Compound	υ(O–H)	υ(COO) asym.	υ(COO) sym.	υ(Sn–C)	υ(Sn–O)
Ligand	3771	1613	1329	–	–
Ph_3SnL	–	1539	1325	535	446
Bu_3SnL	–	1541	1321	537	445
Me_3SnL	–	1546	1322	558	445

Table 3
^{1}H NMR spectral data (δ, ppm) of ligand and complexes.

Compound	O—H	N—H	C—H aromatic	C—(2)H aliphatic
Ligand	9.24	8.73	7.55–7.78	3.89
Ph_2SnL_2	–	8.71	7.49–7.81	3.85
Bu_2SnL_2	–	8.67	7.49–7.82	3.82
Me_2SnL_2	–	8.69	7.36–7.75	3.87

Table 4
^{13}C NMR spectral data (δ, ppm) of ligand and complexes.

Compound	C=O amide	C=O acid	C—H aromatic	C—H_2 aliphatic	^{119}Sn
Ligand	165.32	170.43	127.74–131.55	42.65	–
$PhSnL_3$	160.22	165.26	126.86–133.76	41.64	−120.31
$BuSnL_3$	160.24	166.14	126.25–131.94	42.13	−122.25
$MeSnL_3$	160.31	164.19	127.4–131.44	41.38	−125.52

due to a $CH_2CH_2CH_2CH_3$ group. The aromatic protons in Ph–Sn appear at 7.06–7.17 ppm [19].

Table 4 shows the most relevant ^{13}C and ^{119}Sn NMR data. Because of the poor solubility of the ligand and its complexes in $CDCl_3$, their spectra were recorded in [$^{2}H_6$] DMSO. The C=O resonance group of the complexes at δ 160.22–160.31 ppm were shifted down from the position in the free ligand, which appeared at δ 165.32 ppm. The shift was probably due to a decrease in electron density at the carbon atoms when oxygen is bonded to metal ion [18]. This observation lends further credibility to the conclusion that complexation occurs through the oxygen atoms of the carboxylate group. Ph_2SnL_2, Bu_2SnL_2 and Me_2SnL_2 showed resonance at δ −442.86, −436.83 and −431.49 ppm, respectively, which is well within the range for six-coordinated complexes. In Ph_2SnL_2, ^{119}Sn resonance appeared, as expected, at a lower field region than in Bu_2SnL_2 and Me_2SnL_2, despite the greater electron withdrawing capability of the phenyl group. The resonance at δ (−442.86 ppm) probably reflects the greater shielding ability of the phenyl group.

The ultraviolet visible electronic spectrum of the ligand benzamidoalanine in DMSO solvent is shown in Table 5. As expected, the complexes showed different absorptions from that of the free ligand, with bands shifted to different wavelengths. On the basis of these findings, the suggested structure of the complexes is that shown in Fig. 1.

Table 5
Electronic spectra for ligand and its complexes.

Compound	Absorption bands (nm)		
Ligand	282	301	328
$PhSnL_3$	328	328	340
$BuSnL_3$	296	287	334
$MeSnL_3$	280	290	300

3.2. Antimicrobial activity

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems, comprising about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms are particularly vulnerable to man's selfishness for survival, who has sought to deprive them of their habitat by using antimicrobial agents. These microorganisms

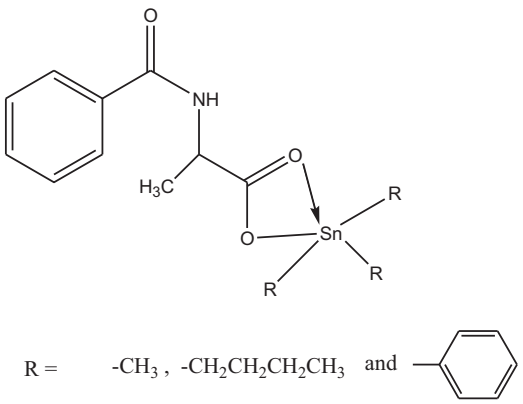

Fig. 1. Deduced structure of organotin complexes with benzamidoalanine.

Table 6
Effects of synthesized compounds on human pathogens in vitro.

Compound	Activity zone of inhibition in mm (MIC in μg/mL)							
	S. aureus	*S. epidermidis*	*M. luteus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*A. niger*	*A. fumigatus*
$PhSnL_3$	24 (17.3)	22 (20.9)	26 (17.2)	21 (19.3)	23 (21.8)	25 (22.6)	27 (19.0)	24 (9.1)
$BuSnL_3$	21 (10.7)	19 (28.4)	20 (25.6)	23 (21.6)	19 (24.2)	27 (20.2)	26 (22.8)	25 (21.8)
$MeSnL_3$	25 (10.2)	22 (12.6)	22 (22.4)	23 (27.4)	20 (32.8)	23 (30.2)	21 (34.2)	20 (31.2)
Ciprofloxacin (100 μg/disc)	29 (0.2)	31 (0.39)	30 (0.1)	29 (0.3)	32 (0.2)	3 (0.25)	33 (0.1)	–
Ketoconazole (100 μg/disc)	–	–	–	–	–	–	30 (6.1)	29 (0.23)
Dimethylformamide	–	–	–	–	–	–	–	–

have responded by developing resistance to this offensive. Currently, antimicrobial resistance among bacteria, viruses, parasites and other disease-causing organisms is a serious threat to infectious disease management globally [20–23].

Antimicrobial agents act selectively on vital microbial functions, with minimal or no effect on host function. Different agents act differently; their mechanisms of action can be categorized on the basis of their structure or on the function that they affect, i.e. inhibition of cell wall synthesis, of ribosome function, of nucleic acid synthesis, of folate metabolism or of cell membrane function. The resistance mechanisms therefore depend on which pathways are inhibited by the drugs and whether the organisms can modify those pathways [24–26]. Resistance is either intrinsic or acquired. Microorganisms with intrinsic or natural resistance either do not have target sites for drugs or have low permeability to drugs because of the chemical nature of the drug or the microbial membrane structure. Acquired resistance, in which a naturally susceptible microorganism acquires ways of not being affected, can occur in various ways.

The synthesized compounds were moderately active against the tested microorganisms, with ranges of MIC values of 3.4–29.4 μg/mL for *S. aureus*, 2.1–28.2 μg/mL for *S. epidermidis*, 1.2–28.7 μg/mL for *M. luteus*, 2.0–27.7 μg/mL for *B. cereus*, 3.1–32.8 μg/mL for *E. coli*, 2.4–36.2 μg/mL for *P. aeruginosa*, 1.1–34.2 μg/mL for *A. niger* and 1.7–31.8 μg/mL for *A. fumigatus*. $MeSnL_3$ was the most potent antimicrobial agent, with MICs of 3.4, 2.1, 1.2, 2.0, 3.1, 2.4, 1.1 and 1.7 μg/mL against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *A. niger* and *A. fumigatus*, respectively. $MeSnL_3$ had significantly greater antimicrobial activity than the standard drugs ciprofloxin and ketoconazole. The potent antibacterial and antifungal activity of $MeSnL_3$ might be due to the presence of a strong electron-withdrawing substituent, with two nitro groups on the benzylideneamino moiety of the 1,3,4-thiadiazole. Compounds containing weak electron-withdrawing groups, like bromo and chloro, and electron-donating groups like methoxy, methyl and hydroxy groups, had weak antimicrobial activity (Table 6).

4. Conclusion

The antimicrobial activity of the synthesized compounds may be due the presence of a versatile pharmacophore, which might increase the lipophilic character of the molecules and facilitate passage across the membrane of the microorganism. $MeSnL_3$ was the

most potent antimicrobial agent when compared with ciprofloxin and ketoconazole.

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